Evaluating tolerance to NaCl in *Vitis vinifera* ssp. *sylvestris* by *in vitro* culture of buds.

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Abstract

The cultivation of the vine is subjected to significant genetic erosion driven by the introduction of foreign vine varieties, commercially more interesting. This decrease in biodiversity dangerously enhances the vulnerability of these vineyards situations, hitherto unknown and expected due to global change, such as pests, diseases or sensitivity to abiotic stresses, increasing saline soils. *In vitro* plant tissue culture allows to obtain a large number of healthy plants, both genetically and physiologically homogeneous. Moreover, being a closed system, plants can be selected according to their response to several factors including salt stress. In this work the *in vitro* bud culture is used to compare, according to their salt tolerance (NaCl), plants of two Andalusian wild grapevine populations, CO7 and J3, and the rootstock Ramsey considered very tolerant to salt. The results show a good response of explants from the three accessions to the micropropagation system used with a high survival percentage, always superior than 93%, in conditions without salt. On the other hand, the parameters analyzed along the study allow establishing the tolerance threshold at 2.5 gL^{-1} NaCl. CO7 shows higher carotenoids contents and lower MDA level than J3 and Ramsey. The results indicate that it is possible to classify the accessions according to salt tolerance as CO7>J3>Ramsey. And support the *in vitro* culture as a suitable method for early assessment of saline effects on plant material vine.

1. Introduction

The natural salinity of soil together with the salinization caused by the cultivation and/or climate changes have influence on the agronomic performance of crops that are worsening over time. Consequently, salinity is one of the most important problems affecting irrigated agriculture in arid and semi-arid areas (Maas and Hoffman, 1977). Christiansen (1982) indicated that of all the land available for agriculture in the

world (about $14x10^9$ ha), approximately $1x10^9$ ha are affected by salt excess. This surface stands for 10% of soils in the world (Rhoades et al., (1992). These authors indicated that between 20 and 50% of areas under irrigation was salinized. In Spain, the area affected by salt is close to 80,000 km^2 (Szabolcs, 1985). The excess of different ions responsible of soil salinity can decrease the development of plants (Grattan and Grieve, 1992) resulting in a reduction in yield and quality of crops, with important economic losses. At the same time, the land becomes unproductive, with its consequent abandonment and desertification (Royo and Aragües, 1989).

Some agricultural activities, especially the use of saline water for irrigation or overexploitation of aquifers in coastal areas that allow the intrusion of seawater, greatly increase the probability of soil salinization (FAO, 1993).

Differences in salinity tolerance among varieties within a determined crop species are small, due to the selection has been made in non-saline conditions looking for production and quality features only. Consequently, the genetic traits of tolerance are disappearing. However, in many wild species, where natural selection has taken place in saline habitats, variations in salt tolerance are often found.

The effects of salt are generally attributed to Na^+ and Cl^- levels (Troncoso et al., 1999; Cantos et al., 2002; Ashraf and Harris, 2004). Plant tolerance to saline soil is regulated by different mechanisms. Osmotic adjustment is the accumulation of solutes such as sugars, glycerol, sorbitol and proline in the cytoplasm of cells to maintain constant gradient water inlet, thereby decreasing the water potential in root (Gale et al., 1967; Termaat et al., 1985). Transpiration decrease by stomatal number reduction and closure, increasing the thickness of the cuticle (Papadopoulos and Rending, 1983; Chaves et al., 2010) or developing hair or laminas on the cell surface, which hinder the exchange of water vapor (Marchner, 1986; Cano, 1991).

The term *"in vitro"* plant tissue culture, includes a set of techniques that allow the development of plant material (plants, seeds, embryos, organs, cells and protoplasts) on a nutrient medium, in aseptic environment, in a closed space and with controlled environmental conditions (Pierik, 1990). This technique is a useful tool for studies of tolerance due to the easy evaluation of a particular factor, in this case the salt of the medium, keeping constant the rest of factors. These considerations are of great value in breeding programs (Oliveira and Santos, 2011). On the other hand, *in vitro* culture is a

good complement to the *ex vitro* studies, because the plant usually shows similar responses to those presented in greenhouse or field (Cavagnaro et al., 2006; Hamrouni et al, 2008).

According to the OIV (2013), the cultivation of the grapevine spans five continents occupying 7.5 million ha. About 14% of all the world vineyards are located in Spain being the third in production. The wine sector accounts for approximately 5.8% of Spanish final agricultural production.

Grapevine is considered as medium tolerant to salt stress (Mass and Hofmann, 1977). However, various rootstocks and varieties have been demonstrated a wide variability in response to salt, both in pot and field assays (Troncoso et al., 1999; Walker et al., 2002). Ramsey variety is one of the most used rootstocks in the Australian and Californian viticulture for its high tolerance to saline soils, great vigor (Walker et al., 2010), resistance to phylloxera and soil nematodes (Walker et al., 1998).

Wild grapevine (*Vitis vinifera*, L. ssp *sylvestris* (Gmelin)), is a dioecious relative of the cultivated, hermaphrodite grapevine taxon (*Vitis vinifera* L. ssp *sativa)* appeared by mutation and selected by man for its great commercial interest for the production of grapes for fresh consumption, raisin or wine. Wild populations still have a significant overall genetic diversity (Grassi et al., 2003) because they have evolved free from human artificial selection made since the beginning of viticulture. In consequence, the wild grapevine can be useful in improving crop varieties for table, wine or rootstocks, giving greater resistance to certain pests, diseases and abiotic stresses, such as soil salinity. Some of the interesting features of wild grapevines can be transferred through traditional breeding to commercial varieties and rootstocks and thus increase the level of diversity (Ocete et al., 2007).

The *in vitro* response of the wild grapevine to high salt levels has been scarcely studied. Gallardo (2005) and Ocete et al. (2007) working with individuals from 13 populations of wild Andalusian grapevine, found that the alterations produced above the level of NaCl 3 g L^{-1} are caused by water imbalances due to changes in cell osmotic potential and by the toxic effect of high Na^{+} levels. They also indicate that *in vitro* culture conditions can benefit salt tolerance, due to the existence of high relative humidity in the culture vessel that allows maintaining a higher degree of hydration for the

plant. Works with *Vitis vinifera* L. under saline conditions indicate that tolerance to NaCl *in vitro* is variety depending (Barlass and Skene, 1981) and that the selection *in vitro* of grapevine tolerant to salinity needs to be proven in external conditions (Skene and Barlass, 1988). Troncoso et al. (1999) classified by *in vitro* technique eleven grapevine varieties according to their salt tolerance (from 0 to 155mM NaCl). Different authors (Singh et al., 2000; Charbaji and Ayyoubi, 2004) found a significant decrease in total chlorophyll of grapevine leaves growing in increasing salt levels under *in vitro* conditions. Hamrouni et al. (2008), working with commercial and local Tunisian varieties found 80 mM NaCl as the threshold where the plants showed signs of necrosis due to salt, although the behavior was very dependent on the variety. Oliveira and Santos (2011) reported that plants of Baga variety reduced, under 100 mM NaCl, its relative growth rate and leaf area. In all cases the reviewed authors emphasized the culture *in vitro* as a good simulation of the salt effects on field conditions.

The objective of the present study is to compare, using the *in vitro* culture technique, the salt tolerance levels of individuals of two populations, tolerant and sensitive to salt stress, of Andalusian wild grapevine with individuals of the Ramsey rootstock.

2. Material and Methods

2.1. Plant material.

The plant material comes from the *in vitro* gene bank existing in the Institute of Natural Resources and Agrobiology of Seville (IRNAS-CSIC). Three accessions were selected for study: The wild grapevines from southern Spain 23/Guarromán/2 (hereinafter J3) chosen to be sensitive to salt and 14/Montoro/3 (hereinafter CO7) with proven salt resistance (Ocete et al., 2007). The hybrid rootstock Ramsey was used for comparison with the two wild grapevines.

2.2.Experimental design.

2.2.1. Micropropagation of individuals J3, CO7 and Ramsey.

In order to obtain enough material for testing salinity, individuals of J3, CO7 and Ramsey were micropropagated. Homogeneous explants (1 cm and 1 bud) from plants of each accession were placed individually under laminar flow cabinet (Telstar AH-100) into sterile test tubes (21 x 150 mm) with 10 ml of the medium VID described by Troncoso et al. (1990), modified to include 0.072 mgL^{-1} of Benzylaminopurine and

0.024 mgL^{-1} of Naphtalen acetic acid as growth regulators, 20 gL^{-1} of sucrose as carbon source, 100 mgL^{-1} of inositol and 1 mgL^{-1} of thiamine as vitamins and 0.6% of agar. Media were previously sterilized at 120°C and 1 atm pressure for 20 minutes. After sowing the tubes were sealed with polypropylene caps and parafilm and placed in a culture chamber at 24 ºC, with a light intensity of 120 $\mu Em\text{-}2s^{-1}$ and a photoperiod of 16 h of light in order to allow their mixotrophic growth.

2.2.2 Salinity tolerance.

Seventy-two explants per accession were transferred individually to similar test tubes containing individually 10 ml of the same culture medium VID described above with five different NaCl treatments: 0, 1, 2.5, 4 and 8 gL^{-1}, which represent electrical conductivities (Cε) of 2.13; 3.45; 5.44; 7.43 and 12.72 mS/cm respectively. In consequence, 1080 explants (5 treatments x 72 tubes x 3 accessions) were assayed.

2.3. Biometric parameters.

After 45 and 60 days of *in vitro* culture the number of dead plants, stem length, bud and shoot numbers per plant and root development of plantlets were determined in relation to the saline treatment. Fresh weight was quantified at the end of experiment (60 days) removing and weighing separately the stem and root of each explant on a precision balance, Mettler 400 PJ. Once determined the fresh weight, stems and roots of each plant were placed in an oven for 72 hours at 60°C. After that, dry weight using a precision scale Cobos CB Complet was determined. The hydration level was calculated by the formula: %H=[(fw-dw/fw]100, (fw= fresh weight; dw=dry weight). The relative growth rate (RGR) (Villar et al., 2004) of the stem was calculated using to the formula: RGR ($g \cdot g^{-1}$ $day^{-1)}$ = (ln Bf - Bi ln) · D^{-1}, Bf = final dry biomass; Bi = dry initial biomass (average of 5 explants per treatment dried under the exposed conditions at the beginning of the experiment) and D = duration of the experiment in days.

2.4 Biochemical parameters.

2.4.1. Pigments

At the end of experimental period, photosynthetic pigments were extracted in partial darkness from expanded leaves of plants growth under each treatment. Three samples (50 mg) of fresh plant material per treatment and accession were frozen in

liquid nitrogen and conserved at -80ºC until use. Each sample was extracted in 10 ml of 80% aqueous acetone. After filtering, 1 ml of the suspension was diluted with a further 2 ml of acetone, and chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Cx+c) contents were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd,Japan), using three wavelengths (663, 646 and 470 nm). Pigment concentrations were calculated according to Lichtenthaler (1987).

2.4.2 Cell membrane integrity - malondialdehyde (MDA).

The membrane integrity was estimated from the concentration of malondialdehyde (MDA), which was determined according to the method of Dhinsa and Matowe (1981). First, 0.25 g of tissue were weighed, frozen in liquid nitrogen (NL), and crushed in a cold mortar. Five mL of trichloroacetic acid (TCA) 0.1% (w/v) was then added to the powder and centrifuged at 10000xg, 10 min. After this time, the 1 ml of supernatant was collected, mixed with 4 ml of 0.5% thiobarbituric acid (TBA) (w/v) in TCA 20% (w/v) and heated at 95°C 30 min. After that, it was rapidly cooled on ice and centrifuged again at 10000xg 10 min. Absorption at 532 and 600 nm of the supernatant was determined in a spectrophotometer, using TBA solution as blank. The concentration of MDA in the extracts was calculated using as molar extinction coefficient of 155 mM^{-1} cm^{-1} as follows:

MDA equivalents (nmol ml^{-1})=(Abs_{532}-Abs_{600})/155000x10^6

MDA concentration was expressed as nmol. $(gfw)^{-1}$

2.5. Statistical treatment.

The results obtained have been treated by the statistical software package SPSS Statistics V.22. With the same estimators centralization, dispersion and comparison of means through calculation were calculated using ANOVA Tukey test. The comparison between percentages was performed using the z test (Student t test) using the Bonferroni correction. The degree of relationship between the different variables was determined by the correlation coefficient r and significance tables from Lamotte (1976).

3. Results.

3.1 Survival.

The percentage of survival in control plants (with development of stem and/or root) after 45 days of culture was very high, more than 92%, in the three accessions. However, the survival percentage of J3 explants reached 92.9% (table 1), statistically lower than the others ($p \leq 0.05$). At the end of the experiment (60 days) the percentage of surviving plants was similar and higher than 94.6% in all cases.

After 45 days, the treatment with 1 gL^{-1} NaCl caused more mortality in Ramsey explants. When salt concentration was increased to 2.5 gL^{-1} NaCl, explants of Ramsey and CO7 were the more affected and at higher salt concentrations the mortality of explants of Ramsey and J3 was similar and lower than CO7 ($p \leq 0.05$) (table 1). This accession reached a survival of 100% and 57.7% at 4 and 8 gL^{-1} NaCl, respectively, at the end of experiment.

3.2 Stem and root development.

A high development of stem corresponding to the CO7 accession was observed in the medium without NaCl at 45 days of culture (7.32 cm) ($p \leq 0.05$) (Table 2). This better development continued after 60 days where the explants of CO7 and Ramsey reached 8.14 and 8.88 cm respectively while those J3 only attained 2.61 cm ($p \leq 0.05$). On the other hand, the average of bud and shoot numbers per plants had the same trend. The presence of NaCl in the medium also affected the *in vitro* development of stem in the surviving plants. Explants of J3 developed longer stem at 1 gL^{-1} NaCl at 45 days but, at 60 days, stems of CO7 were the longest. Explants of Ramsey were longer ($p \leq 0.05$) than the other two accessions at 2.5 gL^{-1} salt. Explants of CO7 reached higher stem length and bud number than those of Ramsey and J3 ($p \leq 0.05$) at 60 days of culture in 4 gL^{-1} NaCl. On the contrary, at 8 gL^{-1} CO7 explants had lower stem length and shoot number than J3 and Ramsey ($p \leq 0.05$) (Table 2).

Explant of all accessions developed root system in all salt concentration (table 3). In absence of NaCl, explants of J3 showed the lowest rooting percentage ($p \leq 0.05$) with 84.3% and 85.7% at 45 and 60 days respectively. Rooting of Ramsey explants decreased significantly in relation to J3 when the salt level was increased to 1 and 2.5 gL^{-1} of NaCl. With 2.5 g L^{-1}, the rooting percentage of Ramsey was similar to CO7. On the contrary, at the higher salt concentrations tested, 4 and 8 gL^{-1}, the explants of CO7

reached the highest ($p \leq 0.05$) percentage of rooting whit all accessions overcoming the 52% and he rooting threshold for all of them at 4 gL^{-1} NaCl.

J3 plants presented higher (34.62) ($p \leq 0.05$) root number average in the treatment without NaCl after 60 days of culture, than plants of the other accessions, without differences between Ramsey and CO7. At 1 gL^{-1} salt level, a higher root number was found in Ramsey and J3 (table 3) at 45 and days of culture but not at 60 days when Ramsey fell below J3 and CO7 ($p \leq 0.05$) with an average of 26.5 roots per plant. At 2.5 gL^{-1}, the J3 plants presented a higher number of roots at 45 days and at 60 days again Ramsey had fewer root number ($p \leq 0.05$). When 4 gL^{-1} NaCl were added the explants of Ramsey and CO7 presented more roots than J3 both at 45 and 60 days. In the highest level of salt no statistical differences were found for accession or sampling dates either. In the control without NaCl the average root length was lower (5.83 cm) ($p \leq 0.05$) (table 3) in J3 plants. For the lowest saline level, plants of Ramsey showed shorter roots compared to CO7 and J3. On the other hand, at 2.5 gL^{-1} roots of J3 was statistically higher than both Ramsey and CO7. After 60 days of culture at 4 gL^{-1} the root length of Ramsey explants reached 3.80 cm, significantly higher ($p \leq 0.05$) than CO7 (3.02 cm) and J3 (1.95 cm). In the level of 8 gL^{-1} no differences were found.

3.3 Tissue water contents.

For the control (0 gL^{-1} NaCl) the stem water content was higher ($p \leq 0.05$) (table 4) in the Ramsey and CO7 explants, above 89.3%, compared to J3 with only 84.18%. Root water content of Ramsey explants, slightly higher to 90%, was similar to CO7 and, in both cases, significantly ($p \leq 0.05$) higher than J3, 86.24%. In consequence, the water content of Ramsey plants was higher (91.30%) than CO7 (close to 90%) and both higher than J3 (86.5%). The same trend was found for the relative growth rate (RGR) average: 0.017, 0.012 and 0.032 for Ramsey, CO7 and J3 explants respectively with statistic differences in all cases. At 1 gL^{-1} NaCl the stem water content was higher in the Ramsey and CO7 plantlets, close to 87%, than in J3 with 82.28%. On the contrary, the root water content of the three accessions was similar and around 89%. As result, the Ramsey and CO7 plants had significantly higher water content (88.09%), in relation to J3 (87.75%). In this salt level the RGR was very similar in the studied accessions, 0.035 g g^{-1} day^{-1}. At 2.5 gL^{-1} NaCl, no significant differences were found among accessions in relation to stem water content. Roots of J3 plants reached a higher hydration level ($p \leq 0.05$) (table

4), 86.8%, in relation to Ramsey and CO7, 84 and 80.7% respectively. These differences in root water contents did not influence neither in plant water contents nor in RGR, similar in all cases. Roots from Ramsey accession reached a water content of 87.5% when they were cultivated at 4 gL^{-1} of NaCl, higher ($p \leq 0.05$) than J3 and CO7. No stem growth was observed at this concentration and at 8 gL^{-1} NaCl (table 1).

3.4 Biochemical parameters

Due to the absence of growth at 4 and 8 gL^{-1} of NaCl, photosynthetic pigment and MDA determinations were only quantified in the three levels showed in table 5.

The Chl a and Chl b concentrations were similar in all accessions in the same saline treatment. However, carotenoids concentrations significantly decreased ($p \leq 0.05$) upon exposure to increasing NaCl in plants of Ramsey and J3.

For MDA, in the treatment without salt, the leaves of Ramsey accession presented a high MDA content, 0.46 nmol $(g\ fw)^{-1}$, similar to J3, 0.26 nmol $(g\ fw)^{-1}$, and much higher ($p \leq 0.05$) than CO7 (0.01 nmol $(g\ fw)^{-1}$). The same trend for the lowest saline concentration was observed. For 2.5 mgL^{-1} of NaCl, no differences between plants of Ramsey and J3 were found (0.37 nmol $(g\ fw)^{-1}$), however there was a lower level ($p \leq 0.05$) of MDA in J3 leaves (0.18 nmol $(g\ fw)^{-1}$).

4. Discussion

From the exposed results it is possible to highlight the good response of explants from the three accessions to the culture medium, with a high survival percentage, always superior than 93%, in salt absence. This behavior of grapevine explants with this medium is well known (Troncoso et al., 1990; 1999; Maghradze et al., 2015). Only 1 gL^{-1} NaCl was enough to differentiate among accessions. In this salt level and at 2.5 gL^{-1} NaCl, survival of wild grapevine accessions was slightly better than Ramsey rootstock, but in the higher salt concentrations always CO7 explants were the most tolerant with a survival superior to 53% in any case, both at 45 and 60 days. On the contrary, J3 and Ramsey explants never reached 15% of survival plants in the highest salt concentration.

The presence of NaCl in the medium also affected the *in vitro* development of the surviving plants. For all selected criteria (stem growth, number of buds, number of

shoots) an inverse relation with the salt concentration was observed in the all accessions ($r = -0.8420$; $r = -0.8554$; $r = -0.81172$ respectively) and specially ($p \leq 0.05$) for rooting, number of roots and length of roots, ($r = -0.8973$; $r = -0.9725$; $r = -0.9896$, respectively). The magnitude of the effects differed among grapevine accessions, but without clear differences. Taking into account the results, it is difficult to classify the studied accessions in relation to salt tolerance according to the number or length of roots for the high variability of these parameters among accessions in a determined salt level.

The water content in the treatment without salt was significant lower than the others, as a consequence of the lowest RGR of this accession. Thus, in agreement with Troncoso et al. (1999), the tissue water content of stressed grapevine *in vitro*, seems to be another suitable parameter for testing their salt tolerance.

Decreases in chlorophylls a and b in relation to salt contents in the medium were no significant. However, the carotenoids contents in relation to NaCl levels showed differences between treatments and accessions. The carotenoids, together with other organic solutes as proline and soluble sugars, are considered protectors against salt damages (Fozouni et al., 2012). Moreover, reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions (Fozouni et al., 2012).

Salinity affects the strength of the forces bringing the complex pigment protein-lipid, in the chloroplast structure. As the chloroplast is surrounded by a membrane its stability is dependent on the membrane stability (Yeo et al. 1990, Ali et al. 2004). The higher increase of carotenoids content in plants of CO7 may indicate that this accession has a better ability to protect plant from photo oxidation. Carotenoids accumulation in response to salt stress has been reported for pineapple (Aziz et al., 2011), banana (Haq et al., 2011) and grape (Fozouni et al., 2012). Determining the MDA content and hence, the extent of membrane lipid peroxidation, has often been used as a reliable tool to assess the degree of plant sensitivity to oxidative damage. (Blokhina et al., 2003). Data of this work showed a remarkable increase in shoot MDA content for J3 and Ramsey than CO7 accession at the different NaCl concentrations. Considering MDA content in shoots, lipid peroxidation was significantly higher in J3 and Ramsey accessions under 1 and 2.5 gL^{-1} NaCl treatments than in CO7 plants, then the accessions Ramsey and J3 are more sensitive to salt.

5. Conclusions

The present study was conducted to evaluate, by *in vitro* culture, the response to increasing salt concentrations (0, 1, 2.5, 4 and 8 gL-1 NaCl) of plants of two wild grapevine accessions (CO7 and J3) and a rootstock (Ramsey) considered very tolerant to salt stress. The results obtained show a salt dependent variability according to the accession used and salt levels. On the other hand, the parameters analyzed along the study constitute good indicators of vitroplants ability to tolerate salt stress. Biometry values, leaf water contents, relative growth rate, chlorophyll contents in plants of the three studied accessions were decreased by salt treatments, establishing the tolerance threshold at 2.5 gL^{-1} of NaCl. CO7 showed the highest carotenoids contents. MDA contents in J3 and Ramsey were higher than in CO7. The results showed that it is possible to establish a classification in relation to salt tolerance as CO7>J3>Ramsey. In consequence, CO7 wild grapevine could constitute an interesting rootstock depending of its phylloxera tolerance.

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Table 1.- Effect of NaCl on *in vitro* survival of explants of the considered accessions.

NaCl (gL^{-1})	Survival (%)					
	45 days			60 days		
	J3	**Ramsey**	**CO7**	**J3**	**Ramsey**	**CO7**
0	92.9 Ba	98,6 Cb	98,6 Bb	94.6 Ba	100 Ca	98.6 Ba
1	98.50 Bb	58,00 Aa	89,70 Bb	100,00 Bb	82,60 Ba	94,10 Ba
2.5	93.80 Bb	82,90 Ba	71,00 Aa	93,80 Bb	94,30 Cc	79,70 Ba
4	81.70 Ba	80,3 Ba	95,7 Bb	87,3 Ba	87,3 Ba	100,00 Bb
8	14.1 Aa	13,9 Aa	53,5 Ab	14,1 Aa	13,9 Aa	57,70 Ab

In each column, means followed by the same capital letter are not statistically different ($p \leq 0.05$) for treatments. In each row, means followed by the same lower case letter are not statistically different ($p \leq 0.05$) for accessions within the same treatment and date.

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Table 2.- Average values of aerial part obtained in *in vitro* culture under increasing salt concentrations.

NaCl (gL^{-1})	Stem Length (cm)						Bud Number						Shoot number					
	45			60			45			60			45			60		
	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7
0	1,63 Ba	5,71 Cb	7,32 Bc	2,61 Ba	8,14 Cb	8,88 Cb	2,57 Ba	4,22 Cb	7,82 Bb	3,87 Ba	6,39 Cb	11,10 Bc	0,59 Ba	1,08 Cb	1,76 Bc	0,94 Ca	1,32 Ca	2,69 Cb
1	0,77 Bb	0,30 Aa	0,32 Aa	2,82 Ba	1,98 Ba	7,47 Cb	0,91 Bb	0,33 Aa	0,49 Aab	2,70 Ba	2,35 Ba	7,88 Bb	0,32 Ba	0,20 Aa	0,19 Aa	0,44 Ba	0,42 Ba	1,35 Bb
2.5	0,27Aa	0,99 Bb	0,05 Aa	1,37 Aa	1,75 Ba	1,46 Ba	0,52 Bb	1,31 Bc	0,07 Aa	1,66 Ba	2,47 Ba	1,91 Aa	0,25 Bb	0,66 Bc	0,04 Aa	0,34 Ba	0,77 Bb	0,30 Aa
4	0,06 Aa	0,02 Aa	0,14 Aa	0,09 Aa	0,04 Aa	0,49 BAb	0,20 Aa	0,04 Aa	0,21 Ab	0,30 Aa	0,08 Aa	0,89 Ab	0,10 Aa	0,04 Aa	0,09 Aa	0,15 Aa	0,08 Aa	0,23 Aa
8	0,05 Ab	0,05 Ab	0,00 Aa	0,05 Ab	0,05 Ab	0,01 Aa	0,15 Aa	0,15 Aa	0,00 Aa	0,15 Aa	0,15 Aa	0,00 Aa	0,10 Ab	0,10 Aa	0,00 Aa	0,10 Ab	0,10 Ab	0,01 Aa

In each column, means followed by the same capital letter are not statistically different ($p \leq 0.05$) for treatments. In each row, means followed by the same lowercase letter are not statistically different ($p \leq 0.05$) for accessions within the same treatment.

Table 3.- Average values of root system obtained in *in vitro* culture under increasing salt concentrations.

NaCl (gL^{-1})	Rooting (%)						Root number						Root Lenght (cm)		
	45			60			45			60			60		
	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7	J3	Ramsey	CO7
0	84,30 Ba	98,60 Cb	98,60 Bb	85,70 Ba	100,00 Cb	98,60 Bb	35,52 Ca	32,04 Ca	35,00 Ca	34,62 Ba	35,17 Ca	40,40 Ca	5,83 Ba	6,39 Cb	6,95 Cb
1	93,90 Bb	49,30 Ba	88,2 Bb	96.30Bb	64.70 Aa	91.50 Bb	23,71 Bc	15,24 Bb	9,37 Aa	42,80 Bb	26,53 Ba	42,87 Cb	6,30 Bab	5,38 Ca	6,37 Cb
2.5	89,20 Bb	50,00 Ba	71,00 Bab	90,80 Bb	72,90 Ba	79,70 Ba	15,97 Bb	5,57 Aa	6,73 Aa	31,67 Bb	14,00 Aa	19,22 Bb	5,04 Bb	3,58 Ba	3,35 Ba
4	73,20 Ba	78,90 Ca	95,70 Bb	84,50 Ba	84,50 Ba	95,70 Bb	5,65 Aa	18,20 Bb	15,40 Bb	12,20 Aa	24,02 Bb	18,20 Bb	1,95 Aa	3,80 Bc	3,02 Bb
8	5,60 Aa	5,60 Aa	52,10 Ab	5,60 Aa	5,60 Aa	56,30 Ab	2,25 Aa	2,25 Aa	3,95 Aa	2,25 Aa	2,25 Aa	4,03 Aa	0,25 Aa	0,25 Aa	0,44 Aa

In each column, means followed by the same capital letter are not statistically different ($p \leq 0.05$) for treatments. In each row, means followed by the same lowercase letter are not statistically different ($p \leq 0.05$) for accessions within the same treatment.

Table 4.- Average of water contents (%) of stem, root, complete plants and Relative Growth Rate (RGR) of grapevine accessions 60 d after the onset of *in vitro* culture under increasing concentrations of NaCl in the medium.

	Stem water content (%)			**Root water content (%)**			**Plant water content (%)**			**RGR**		
NaCl (gL^{-1})	**J3**	**Ramsey**	**CO7**	**J3**	**Ramsey**	**CO7**	**J3**	**Ramsey**	**CO7**	**J3**	**Ramsey**	**CO7**
0	84,18A a	89,36 Bb	89,90 Bb	86,24A a	90,25 Ba	89,33A Ba	86,50Aa	91,30 Cc	89,80Bb	0,0032Aa	0,017 Ac	0,012 Ab
1	82,28A a	86,92 Bb	86,92 Bb	89,3 Aa	88,99 Ba	89,04 Ba	87,75Aa	88,09 Bb	88,09Bb	0,034Ba	0,036 Ba	0,035 Ca
2,5	85,39A a	83,81 Aa	85,83 Aa	86,81A b	84,03 Aa	80,73 Aa	86,48Aa	83,94 Aa	82,41Aa	0,033Ba	0,030 Ba	0,029 Ba
4	-	-	-	83,82A a	87,50 Bb	81,22Aa	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-

In each column, means followed by the same capital letter are not statistically different ($p \leq 0.05$) for treatments. In each row, means followed by the same lowercase letter are not statistically different ($p \leq 0.05$) for accessions within the same treatment

Table 5. Chlorophyll a (chl a), chlorophyll b (chl b), carotenoids (xanthophyll plus carotenes) and malondialdehide (MDA) in leaves of studied accessions in response to *in vitro* treatment with increasing ranges of NaCl for 60 days.

NaCl Level (gL^{-1})	**Accessions**	**Chl a** µg gfw^{-1}	**Chl b** µg gfw^{-1}	**x+c** µg gfw^{-1}	**MDA** nmol/gfw^{-1}
0	**CO7**	2,72 A	1,26 A	0,51 B	0,01 A
	J3	2,68 A	1,20 A	0,55 B	0,26 AB
	Ramsey	1,79 A	0,95 A	0,34 A	0,46 B
1	**CO7**	0,87 A	1,55 A	0,17 B	0,26 A
	J3	0,70 A	1,23 A	0,16 B	0,36 B
	Ramsey	1,48 A	2,67 A	0,09 A	0,46 C
2.5	**CO7**	0,67 A	0,88 A	0,09 B	0,18 A
	J3	0,44 A	0,70 A	0,03 A	0,37 B
	Ramsey	0,78 A	1,31 A	0,13 B	0,37 B

In each column, means followed by the same capital letter are not statistically different ($p \leq 0.05$) for treatments